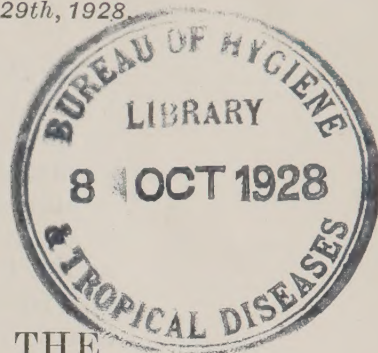


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KALA-AZAR AND ORIENTAL SORE: THE PROBLEM OF TRANSMISSION.*

BY

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THE present-day position regarding the method of transmission of kala-azar, and of the parasitologically allied disease oriental sore, centres around sand-flies of the genus *Phlebotomus*, those minute irritating flies which transmit the febrile condition known as phlebotomus fever, which have long been suspected as being transmitters of oriental sore, and which have comparatively recently fallen under suspicion in relation to kala-azar. It may be said at the outset that though there is very good circumstantial evidence that these flies are the actual vectors of kala-azar and oriental sore, the final proof of experimental transmission has not yet been obtained, though there is every reason to suppose that when an infected sand-fly bites an experimental animal or man himself the parasites are injected into the wound from the proboscis of the fly. The reason for this failure is not apparent, but it may be that unless the vertebrate host used for the experiment is in a particularly susceptible condition the small dose of organisms injected is insufficient to give rise to infection. It can hardly be that the organisms are not virulent, for if a sand-fly which has infected itself by feeding on a case of the disease is dissected and the bulk of the organisms it contains is injected with a syringe into a suitable animal in the case of kala-azar, or into man in the case of oriental sore, infection readily occurs. Though at the commencement of the sand-fly work there appeared to be every promise that the problem of transmission of the two diseases, kala-azar and oriental sore, would be quickly solved, an unforeseen obstacle has arisen in that experimental animals and man fail to contract the disease even when fed upon by large numbers of heavily infected sand-flies. Whether this obstacle will be overcome, or whether it is an indication that, as has happened before in connexion with these diseases, we are on the wrong track, future work alone can disclose.

* Read in opening a discussion in the Section of Tropical Medicine of the Annual Meeting of the British Medical Association, Cardiff, 1928.

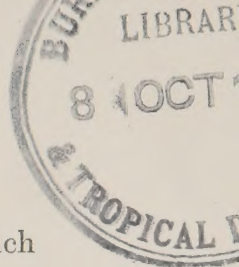
As the problem is still unsolved it will be advisable for us to consider other possibilities. In order to do this it is necessary to have some knowledge of the nature of the parasite which is known by the name "Leishmania," or, popularly, the Leishman-Donovan body. When it was first discovered, in 1903, by the late Sir William Leishman, and again in the same year by Donovan, its exact nature was not fully realized, but Sir Leonard Rogers soon showed by the culture method that it was a flagellate of the herpetomonas or, as I prefer to call it, the leptomonas type. Flagellates of this kind are essentially flagellates of insects and other invertebrates, in which they occur both as the non-flagellated body, like the Leishman-Donovan body, and as the elongate flagellated leptomonas, which, in the case of the human parasite, is hardly, if ever, found in the human body, but which occurs in cultures and in the intestine of certain insects, particularly the sand-fly and bed-bug, which have ingested the small round forms. In all cases in which the life-cycle of an insect leptomonas is known it has been found that infection is spread from insect to insect by means of small round forms, which have the structure of the Leishman-Donovan body and which escape in the faeces of the insect. The common leptomonas of the flea, *Leptomonas ctenocephali*, is a flagellate of this type, and it is spread from flea to flea by such forms which escape in the faeces and are ingested by the flea larvae. In the insect itself the infection in all cases tends to be one of the hind-gut. So essentially are the leptomonas flagellates parasites of insects and other invertebrates, that it is safe to assume that the parasites of kala-azar and oriental sore were originally insect flagellates, like that of the flea, which passed from insect to insect, and that the infection of human beings came at a later period.

It has been suggested that the parasite of kala-azar might pass directly from man to man, and in support of this might be brought forward the observation of Perry, who showed that in kala-azar the villi of the intestine may be packed with macrophages filled with parasites in such a manner that escape into the faeces would seem to be inevitable. Furthermore, it has been shown by Shortt that parasites do actually occur in the urine, so that contamination of water is a possibility. There are isolated observations which indicate that man-to-man infection is a possibility. Thus, Archibald in 1914, in the Sudan, infected monkeys by feeding them with infected spleen material; while Adelheim, in 1924, reported that a healthy mouse kept in a jar for five months with an infected mouse contracted the disease. There is no evidence, however, that the Leishman-Donovan body can survive in water. It certainly cannot assume the flagellate stage in this medium, and, unless we are to ignore entirely the fact that the parasite can develop into a flagellate, we must give up the idea of man-to-man infection as the normal method of spread of the disease.



So important to my mind is the flagellate stage which occurs in cultures and in the intestine of insects, and so characteristic is it of an insect flagellate, that there appear to be overwhelming reasons for believing that the Leishman-Donovan body must have an invertebrate host. Now the mere fact that the Leishman-Donovan body can develop into a flagellate in the stomach of an insect is not in itself proof that such an insect is the host. As first shown by Patton as long ago as 1907 in India, bed-bugs fed on kala-azar cases will ingest parasites which, during the next few days in the stomach of the bug, develop into the flagellate stage. There is, however, no great multiplication in the bug. If few parasites are ingested, few flagellates appear, and vice versa. If the bug had been the true invertebrate host we would have expected that if only a few parasites had been ingested, eventually many flagellates would have developed. Furthermore, the flagellates tend to die out in the bugs, a second feed of blood being particularly fatal to them. In true insect hosts of any flagellate the organisms increase in number progressively and tend to persist for the remainder of the insect's life. Another point of some importance is that in the case of flagellates in the real insect host these show a tendency to attach themselves to the intestinal epithelial cells by their flagellated ends, the whole epithelium being sometimes covered with them. No such attachment occurs in bugs, so that, quite apart from the lack of correlation between the distribution of bed-bugs and kala-azar, there is good reason for believing that the bug is not the vector and that the development into flagellates which occurs in the bug's stomach is a pseudo-development and occurs merely because the stomach of the bug contains a quantity of blood. The development is comparable with that which occurs in blood media in the test tube. I myself in 1911 showed that the parasite of oriental sore would similarly develop in the bed-bug, but no one would suppose that the bed-bug is the vector of this disease. As a matter of fact, trypanosomes, spirochaetes, and other organisms will multiply to some extent and survive for relatively long periods in the stomach of this insect. There is no question of the bug being a vector of these various organisms. I think, therefore, we are justified in ruling out the bed-bug, even though Young has recently stated somewhat casually that he and Hertig were successful on one occasion in effecting transmission of kala-azar from one hamster to another by its means. Nicolle and Anderson in 1925 announced the complete failure of a laborious attempt to transmit the disease from dog to dog by the bed-bug.

In the Mediterranean region, where kala-azar occurs chiefly in the infantile form, not infrequently dogs, and very rarely cats, suffer from the same disease. Basile and other Italian workers very naturally thought that the flea might be a vector, and this idea was strengthened when it was found that fleas taken from infected dogs harboured



flagellates similar to the Leishman-Donovan body and the flagellates which develop from it. It was not at first realized that fleas, as I have already mentioned, are liable to a leptomonas infection which is peculiar to themselves. It was subsequently shown that the Leishman-Donovan body would not develop in fleas, and very careful experiments by myself, Pereira da Silva, and Nicolle and Anderson have failed to effect transmission of the disease from dog to dog by these insects. The flea hypothesis, like that of the bed-bug, has therefore been abandoned, so far as I am aware, by all observers. Numerous other blood-sucking arthropods have been tested, with results even less encouraging than those obtained with bugs and fleas. Even the ancylostomes, which attach themselves to the mucosa of the intestine and suck blood, and thus are very probable ingestors of Leishman-Donovan bodies, have been carefully examined for flagellates or other stages of the parasites, with negative results.

The whole position of the problem of transmission of kala-azar and oriental sore reached a stage when it appeared to be more obscure than ever. Then the interesting discovery that the parasite of kala-azar would develop in the Indian sand-fly *Phlebotomus argentipes* was announced by Knowles, Napier, and Smith, working in Calcutta in 1924. It was also observed by Sinton that there was a relationship between the distribution of this sand-fly and kala-azar in India. The observations were quickly confirmed by Christophers, Shortt, and Barraud with the same sand-fly in Assam, and by Young and Hertig, and Patton and Hindle in North China with sand-flies of that locality. Furthermore, Adler and Theodor in Palestine, and Parrot and Donatien in Algeria, showed that the parasite of oriental sore would similarly develop in sand-flies. The important point about this development was that it was a progressive one, and, unlike the development which had been demonstrated to occur in the bed-bug, a relatively small number of organisms ingested by the fly led to a massive infection of the stomach. Furthermore, the flagellates showed a tendency to attach themselves to the cells, so that in both these respects the organism was behaving in the sand-fly as a flagellate would be expected to do in its true invertebrate host. In one direction, however, there is a marked difference from all known insect leptomonas. As noted above, the infection in the insect tends to be one of the hind-gut, from which small rounded bodies, resembling the Leishman-Donovan body, are voided in the faeces. In the sand-fly infected by feeding on a case of kala-azar or oriental sore no such infection of the hind-gut occurs. On the contrary, the development of the flagellates occurs in the stomach, particularly in its anterior part, and there they show a tendency to attach themselves to cells. Having produced a massive infection of the stomach, the flagellates may extend forwards to the oesophagus and thence into the buccal cavity. Further-

more, there is no tendency towards the production of the small round Leishman-Donovan bodies, which are so characteristically formed in the hind-gut in the case of insect flagellates. Adler has shown that if an infected sand-fly feeds through a rabbit-skin membrane on a sterile fluid, flagellates can afterwards be demonstrated in the fluid, an observation which proves conclusively that the organisms escape from the proboscis when the sand-fly feeds. The development of the flagellates in the anterior part of the alimentary tract is thus not associated with any development in the hind-gut, nor with the passage of small rounded forms in the faeces, though occasionally isolated flagellates occur in the hind-gut, doubtless as a result of having been swept there from the heavily infected stomach. It seems very probable that the parasites of kala-azar and oriental sore originally lived in some insect and gave rise to an infection of the insect type—namely, infection of the hind-gut and passage of rounded forms in the faeces. If this original insect host was the sand-fly, we must assume that the infection in the fly has altered in character from one in the hind-gut to one in the anterior part of the alimentary canal. However, there appears to me to be another possibility. The sand-fly may not be the original host. The flagellate, as the Leishman-Donovan body, having become established in man, it is evident that any blood-sucking insect would be able to ingest parasites by feeding on the human being, as the parasites occur both in the circulating blood and in the skin. If the conditions in the insect were favourable to the development and multiplication of the flagellate it would come about that such an insect would become infected with flagellates.

It may be that the sand-fly is an insect of this type, and that the development which occurs in the anterior part of its alimentary canal is to be explained on the assumption that it has become secondarily infected from human beings, who in the first place acquired the parasites from some insect in which the flagellates lived, as do other insect leptomonas. This does not mean that the sand-fly would be unable to transmit the infection, but it would indicate that if the original hypothetical insect host still exists the virus would be able to maintain itself by direct passage from insect to insect without necessarily infecting man.

It seems to me simpler to regard the sand-fly as a secondary insect host than to admit that an original hind-gut infection has in the same fly changed to one in the anterior part of the alimentary canal. The same difficulty occurs in the case of trypanosomes transmitted by tsetse flies, where an anterior development also occurs, and it is possible that a similar explanation will hold in their case also. If the original insect host still survives, the problem of the eradication of the leishmaniasis would be a difficult one, for there would then be a permanent insect reservoir of the virus.

The remarks which I have just made show that sand-

flies can readily be infected by allowing them to feed on cases of kala-azar or oriental sore. In cases of oriental sore where the lesion is in the pre-ulcerative stage a sand-fly feeding on it readily takes up leishmania, as these parasites occur in myriads enclosed in large macrophages or clasmotocytes just below the epidermis. In the case of kala-azar, the presence of parasites in blood films made from the peripheral blood has been demonstrated, and it has generally been supposed that the sand-flies ingest the parasites in the circulating blood. Observations by Cash and Hu have shown, however, that in experimentally infected hamsters, and even in human beings suffering from the disease, the skin may contain larger numbers of macrophages packed with parasites. They have suggested with good reason that it may be that sand-flies infect themselves from the parasites in the skin, and that in many cases when parasites have been found in films of the peripheral blood these may actually have been derived, in some cases at least, from the skin. Hindle has made some observations which indicate that a relationship exists between intensity of the skin infections in hamsters and the percentage of flies which become infected after feeding on them. Cases of Indian kala-azar may show a peculiar skin eruption, first described by Bramachari in 1922 as dermal leishmanoid. In this condition, which is by no means uncommon, there occur cutaneous nodules harbouring parasites. It would be a relatively easy matter for sand-flies to take up parasites from these lesions as they do in oriental sore.

Not only do flies become infected by feeding on human beings, but also when fed on experimentally infected hamsters, and in mentioning these little rodents I must emphasize the great importance of the discovery of Smyly and Young in 1924, in China, that the hamster is very susceptible to inoculation with the parasite of kala-azar. Before this observation the lack of a susceptible laboratory animal was a serious handicap to kala-azar research. Now the virus can be maintained with ease in the laboratory, and it is a simple matter to feed sand-flies or other insects on the hamsters. It is of interest to note that Young and Hertig reported in 1925 that they had examined 4,480 wild hamsters for the occurrence of natural infection. Out of this number one was found to have a heavy infection. It had come from a locality where 5 per cent. of the inhabitants suffer from the disease. It would not appear, however, that the hamster can in any way be regarded as a reservoir of the virus. In experiments the hamster is much more susceptible to intraperitoneal injections of the virus than to cutaneous ones, and this may explain why workers in India and China have failed entirely to infect hamsters by allowing hundreds of infected sand-flies to feed on them, while the flagellates taken from infected sand-flies and injected intraperitoneally readily produce infection. It is evident that the dose of flagellates injected

into the skin by a large number of sand-flies is, as far as present records show, insufficient to produce infection. It seems that recourse must be had to human experiments, and I understand that these are being carried out with volunteers in India—an experiment which is attended with little danger now that a practically certain cure is available in the various organic antimony compounds when employed in the early stages of the disease. Even these experiments may not at once give results, for the factor of susceptibility of the individual is an important one. Thus Maggiore in 1925 failed to infect human beings by intracutaneous and subcutaneous injection of virus taken directly from cases of kala-azar.

Assuming that sand-flies of the genus *Phlebotomus* are the actual vectors of kala-azar and oriental sore, how can the parasites in the flies be transferred to human beings? From what I have said of their presence in the anterior part of the alimentary tract, and the fact that they escape from the proboscis into a fluid on which an infected sand-fly is feeding, it is legitimate to conclude that they actually enter the wound inflicted by the sand-fly during the feeding act. There are, however, other possibilities. In common with many other blood-sucking insects the sand-fly ejects fluid from the rectum while it is feeding. In the case of the transmission of *Trypanosoma lewisi* from the flea to the rat it is in such ejected droplets that the infective forms of the trypanosome occur. The droplets are licked from the fur by the rat, which thereby becomes infected.

As already noted, occasional flagellates may be found in the hind-gut of the sand-fly as a result of having been washed there accidentally from the stomach, and this being the case it is probable that on very rare occasions flagellates would escape in the droplets ejected from the rectum. The number, however, would be very small, and in any case would hardly be greater than the number injected by the sand-fly from its proboscis. The ejected droplets fall on to the skin while the sand-fly feeds, and the subsequent scratching, as a result of the irritation, might lead to infection of the skin or conveyance of material on the fingers to the mouth or conjunctiva. The dose of organisms would be so small that it hardly seems probable that infection would occur in this way when that injected directly into the skin from the proboscis fails to infect. There is, however, another consideration. The heavily infected sand-fly might be intentionally or inadvertently crushed on the skin, with the result that a very much larger dose of organisms would be deposited. In the case of the transmission of relapsing fever by the louse, it is the fluid from the injured or crushed louse which brings about infection by contamination of the skin or conjunctiva. It has been shown by Adler and Theodor that if a sand-fly infected with the parasite of oriental sore is dissected and the bulk of the organisms it contains is injected into the skin of a human being, infection results, so that in the case of

this disease the crushing of an infected sand-fly on the skin might easily give rise to infection. In the case of kala-azar, however, there is no evidence that the injection into the skin of organisms from an infected sand-fly will produce infection, though it would probably do so in some cases, as hamsters may occasionally be infected by the subcutaneous injection of the flagellates from the culture tube. Hindle and Patton have actually attempted without success to infect hamsters by inoculating flagellates from sand-flies into the skin, by rubbing them into excoriations of the skin, and by introducing them into the mouth. It is possible, however, that under certain favourable conditions the scratching into the skin of a large dose of organisms from a crushed sand-fly or the conveyance on the fingers of such a dose to the mouth or conjunctiva might produce infection. If experiments designed to transmit infection by the bites of infected sand-flies continue to fail, as they have done up to the present, then it appears to me that the other possibilities I have just mentioned will have to be still further investigated.

As regards the particular sand-flies involved in India, it is *Phlebotomus argentipes* which, as already stated, corresponds fairly well in its distribution with that of kala-azar. Another sand-fly, *Phlebotomus papatasi*, has also been infected in Calcutta, but with greater difficulty. Thus of 102 *P. argentipes* fed on kala-azar cases by Napier in Calcutta 43 showed subsequent infections, while of 101 *P. papatasi* only 2 became infected. Shortt, Barraud, and Craighead on one occasion found a naturally infected *P. argentipes* which had been caught in a kala-azar house in Bihar. It is not probable that *P. papatasi* has any significant share in the transmission of the disease. In North China *P. argentipes* does not occur, and in fact there is no knowledge of any sand-flies south of the Yangtse, a distribution which is again in accord with that of kala-azar in this endemic area. Young and Hertig showed that both *P. major* var. *chinensis* and *P. sergenti* var. could be infected by feeding on infected hamsters, the former in their hands more readily than the latter. Patton and Hindle found that both these flies could be readily infected, and in addition infected them by allowing them to feed on human beings suffering from the disease. In the case of *P. major* var. *chinensis* the development of flagellates in the stomach is marked, many of the organisms attaching themselves to the lining epithelium. In many flies they extend forward along the oesophagus to the buccal cavity and down the proboscis. The infection persists in the flies after the blood has been completely digested and disappeared. In the case of *P. sergenti* var., however, though development occurs in the stomach there is no extension forwards, while the infection disappears with the digestion of the blood. It is therefore less likely to be a vector than *P. major* var. *chinensis*. It is thus apparent that as in India *P. argentipes* is a more efficient host than

P. papatasii, so in North China *P. major* var. *chinensis* is more efficient than *P. sergenti* var.

There is as yet no information available regarding the relationship of kala-azar to sand-flies in other endemic centres of the disease, such as the Mediterranean region, Transcaspia, the Sudan and Kenya, the districts around Lake Tchad, and South America. It will be sufficient to state that Adler and Theodor, working in Palestine, have succeeded in producing infections in *P. papatasii* by feeding them on cultures of various strains of leishmania, but I do not think the results so far obtained throw much light on the problem of transmission in the localities I have just mentioned.

Turning now to oriental sore, the sand-fly has long been considered to be a possible vector. In 1912 I described a leptomonas which occurred in sand-flies in Aleppo. This flagellate was not rediscovered till Adler and Theodor found it in 1925 in *P. papatasii* in Jericho, unless the claim of Laveran and Franchini, about which there appears to be some doubt, that they found it in these sand-flies in Italy in 1920, is substantiated. Flagellates from naturally infected sand-flies were inoculated by Adler and Theodor into the skin of human beings. Oriental sore developed, and from these lesions other laboratory-bred sand-flies were infected. The flagellates in these flies were again injected into the skin of a volunteer, who contracted the disease. Previous to this Sergent, Parrot, Donatien, and Béguet in 1921 inoculated into the skin of a human being the crushed up bodies of seven *P. papatasii* which had been sent to Algiers from Biskra, an endemic centre of oriental sore. Though no flagellates were seen in the material inoculated, a typical oriental sore containing leishmania developed. Parrot and Donatien in 1926 found naturally infected sand-flies of this species in Biskra, and succeeded in infecting sand-flies by feeding them on the tail lesions in infected mice. The evidence connecting *P. papatasii* with oriental sore is parallel to that associating *P. argentipes* with kala-azar in India and *P. major* var. *chinensis* with this disease in North China, with the exception that naturally infected sand-flies have not yet been found in the last-named area. Adler informs me that during a visit to Baghdad he has found there, amongst other sand-flies, *P. sergenti*, and that it is also a possible vector of oriental sore as he has succeeded in infecting it. The only evidence of the method of transmission of cutaneous leishmaniasis in South America, where the disease is very prevalent, is afforded by an experiment described by Aragão in 1922. This observer fed some specimens of *P. intermedius* on a human lesion and later inoculated the crushed up sand-flies into the skin on the nose of a dog, which developed a characteristic sore.

Time will not permit of a discussion of the leishmania and leptomonas infections of vertebrates other than man. As already noted, dogs in the Mediterranean area are liable

to suffer from kala-azar, while in various endemic centres of oriental sore these animals not infrequently contract the disease. There is no reason to suppose that the organisms infecting dogs are different from those which produce the diseases in man. Lizards, such as the gecko and chameleon, may be infected with flagellates of the leptomonas type, and at one time in North Africa it was thought that the gecko, on which sand-flies readily feed, might be a reservoir of the virus of oriental sore. Experiments did not support this view, though the recent statement of Young and Hertig that the flagellate from the gecko will infect hamsters and produce lesions similar to those caused in these animals by the virus of Mediterranean kala-azar opens up another possibility—namely, the identity of the gecko flagellate with the parasite of North African kala-azar. Some observers have tried to connect with oriental sore the naturally occurring leptomonas infections of euphorbias and other plants. Strong has even suggested that in Central America, where these plant infections occur, the plant bugs acquire an infection by feeding on the plants, and a certain lizard an intestinal infection by devouring the bugs. Flagellates from the lizard inoculated into the skin of a monkey produced a lesion in which leishmania-like parasites occurred. These and many other aspects of leptomonas infections open up fields of inquiry which still await exploration.

In conclusion, it may be stated that if the sand-fly is not the transmitter of the leishmania infections of man we are as far off the solution of the problem as we ever were. The remarkably intense infection produced in this insect is difficult to regard as an accident, and the mass of experimental evidence is in favour of the view that both kala-azar and oriental sore are transmitted by species of *Phlebotomus*. On account of the failure of experimental animals, on which infected sand-flies have been allowed to feed, to develop the disease, Young and Hertig have begun to doubt if the sand-fly is really the vector of kala-azar in North China. Workers should not, however, be discouraged by such a temporary set-back, and until every channel has been thoroughly explored it would be foolish, in face of all the evidence, to abandon the theory of sand-fly transmission of this disease.

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